AD NUMBER

AD852677

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; APR 1969. Other requests shall be referred to Department of the Army, Fort Detrick, MD 21701.

AUTHORITY

BDRL ltr, 29 Sep 1971

AD

TECHNICAL MANUSCRIPT 529

SENSORY EFFECTS OF RELATIVE HUMIDITY ON THORACIC SPIRACLES OF *AEDES* MOSQUITOES

Elliot S. Krafsur

APRIL 1969

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

17

Reproduction of this publication in whole or in part is prohibited except with permission of the Commanding Officer, Fort Detrick, ATTN: Technical Releases Branch, Technical Information Division, Fort Detrick, Frederick, Maryland, 21701. However, DDC is authorized to reproduce the publication for United States Government purposes.

DDC AVAILABILITY NOTICES

Qualified requesters may obtain copies of this publication from DDC.

Foreign announcement and dissemination of this publication by DDC is not authorized.

Release or announcement to the public is not authorized.

DISPOSITION INSTRUCTIONS

Destroy this publication when it is no longer needed. Do not return it to the originator.

The findings in this publication are not to be construed as an official Department of the Army position, unless so designated by other authorized documents.

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland 21701

TECHNICAL MANUSCRIPT 529

SENSORY EFFECTS OF RELATIVE HUMIDITY ON THORACIC SPIRACLES OF *AEDES* MOSQUITOES

Elliot S. Krafsur

Plant Pathology Division
PLANT SCIENCES LABORATORIES

Project 1B562602A059 **April 1969**

ACKNOWLEDGMENTS

The excellent technical assistance of HMC J. H. Pelphrey, USN, HM 1 R. E. Gary, USN, and Mrs. Doris Hahn is gratefully acknowledged. I thank Drs. W. A. Rowley, C. L. Graham, and J. H. Gilford for critical reading of the manuscript. The kind support of Dr. C. L. Graham, Dr. J. H. Gilford, CDR H. G. Arm, MSC, USN and LCDR A. J. Gaspar, MSC, USN is acknowledged. Mrs. H. J. Krafsur kindly typed and edited the manuscript.

ABSTRACT

The behavior of the thoracic spiracles of *A. triseriatus* and *A. aegypti* was observed in high (90±5%) and low (<5%) relative humidities. Following a 5-minute exposure to low RH, spiracular responses to 1/2% carbon dioxide were reduced in both species, but only *A. triseriatus* showed attenuated spiracular behavior after a 2-minute exposure. Conservative spiracular opening was observed in both unfed and fed mosquitoes. It is postulated that the spiracular response to low RH is a sensory one.

CONTENTS

FIGURE

TABLES

I. INTRODUCTION*

The presence of regulatory valves in the tracheal system of insects presumably arose to facilitate water conservation while meeting respiratory requirements. Although Hazelhoff[1] was apparently the first to suggest that most water loss occurred through transpiration from the trachea, Mellanby[2] experimentally confirmed the hypothesis by showing that factors causing permanent spiracular opening in Tenebrio larvae greatly accelerated water loss. Bursell[3] demonstrated that water loss from Glossina was a linear function of humidity when spiracles were kept open with carbon dioxide; in the absence of this gas, however, rates of water loss were asymptotic in respect to humidity. Moreover, spiracular control of water loss was markedly more efficient in flies with depleted water reserves. Adult Phormia regina lost more weight in a low relative humidity than in a high relative humidity; further, total oxygen consumption was reduced by dry conditions.[4] It was suggested that the effects of low humidity took place through spiracular transpiration. Bursell[5] considered the effects of a xeric environment on water balance in Glossina and concluded that the highly developed powers of water retention in these forms were due largely to spiracular control. As in Glossina, humidity and water balance must play an important though ill-defined role in survival and longevity of mosquito populations.[6,7] Available evidence points to an intrinsic mechanism of spiracular control of transpiration rather than an extrinsic, sensory one.[8-11] This will be discussed further.

Several lines of evidence thus suggest that spiracles are instrumental in water conservation while still responding to the often conflicting demands of respiration. On the other hand, no evaluation of spiracular response to humidity by direct observation exists beyond the efforts of Geigy and Huber[12] to correlate the degree of spiracular opening with ambient humidity. The object of this study is therefore to survey the immediate sensory effects of "high" and "low" relative humidity (RH) upon spiracular behavior in adult female Aedes (Ochlerotatus) triseriatus (Say) and Aedes (Stegomyia) aegypti (Linnaeus). The intrinsic influence of water balance on spiracular behavior will be explored in a subsequent communication.

* This report should not be used as a literature citation in material to be published in the open literature. Readers interested in referencing the information contained herein should contact the author to ascertain when and where it may appear in citable form.

II. METHODS AND MATERIALS

Equipment and methods for observing and recording behavior of the spiracular valves have been described in detail elsewhere[13] and will be noted only briefly here. Thoracic spiracles of adult mosquitoes were observed at a magnification of 97.5X with two horizontally mounted microscopes equipped with incident illumination. Each insect was suspended by its mesonotum with a vacuum (18 to 22 lb./inch^2) maintained through a blunted 22-gauge hypodermic needle; the needle was mounted on a mechanical stage capable of orienting the mosquito in three dimensions. A microclimate of known composition and RH was provided by shunting suitable amounts of dry, compressed air from steel cylinders through flowmeters and a water tank to a gas delivery tube. The latter directed gas flow over the insect from both the right and left anterior-lateral aspects. Gas flow was standardized at 5,000 cc/minute and temperature was maintained at 70 F. RH was measured with Leeds and Northrup electronic sensing elements accurate to ±1.5% RH. Oscillograms of spiracular behavior were obtained with a two-channel pen recorder activated by push-button controls directed through an amplifier. This mechanism allowed an observer three arbitrary positions for translating spiracular behavior into a permanent record for later analysis. A closed spiracle and two magnitudes of opening were recognized. Opening of the first magnitude was defined as partial separation of the spiracular lips commonly for 1/8 to 1/4 of their length. This was in fact the extent of "normal" opening in mosquitoes at rest in air. Opening of the first magnitude was noted as "toD" and expressed as the number of seconds per minute of observation that spiracles were in such a position. Spiracular opening of the second magnitude was defined as complete separation of the spiracular lips and included opening to the widest possible amplitude; this was noted as "toF" and was expressed as the number of seconds per minute of observation the spiracle was in such a position. "toT" was the total number of seconds per minute of observation that spiracles were open and was hence the sum of toD and toF. "Frequency" refers to the number of spiracular openings per minute of observation. The latter were scored only when opening was from the completely closed position. In the present work, toT will be taken as the index of duration of opening, and toF the index of amplitude. The number of openings from the closed position was noted as "frequency."

Exclusively female pupae were allowed to emerge and were maintained thereafter in gallon-sized cardboard containers at 80 F and 80±5% RH. Three cotton pads containing 30 cc of 10% sucrose solution each were provided fresh daily for nourishment.

Mosquitoes were prepared for spiracular observation in the following manner: (i) a mosquito was gently aspirated into a plastic shell vial; (ii) the vial was placed in a freezer at 25±5 F for 1½ to 2 minutes; (iii) the immobilized insect was placed on a flat surface and the right prothoracic leg was amputated to prevent occlusion of the right mesothoracic spiracle; (iv) the mosquito was attached by the mesonotum to a blunted hypodermic needle; (v) the insect and needle were mounted on the mechanical stage; (vi) following recovery from chilling under room conditions, the mosquito

was exposed to an air flow of known RH for a stated period of time, (vii) observation and recording of spiracular behavior was then undertaken for a continuous 3-minute period. The first minute of observation was taken in air alone and the following 2 minutes were taken in air plus 1/2% carbon dioxide.

III. RESULTS

The first series of observations were directed toward evaluation of spiracular behavior in high (90±5%) and low (<5%) relative humidities following a 5-minute acclimatization to the experimental conditions. Two-day-old A. aegypti were subjected at random to one of the relative humidities; a total of 30 insects were observed in each environment. Two-, three-, and four-day-old A. triseriatus were similarly tested. In both A. aegypti (Table 1) and A. triseriatus (Table 2) the average duration of spiracular opening (toT) was less in air of low RH than in air of high RH; however, the differences were not statistically significant. On the other hand, low RH effected a significantly more conservative spiracular response to 1/2% carbon dioxide in both species. Additionally, the proportion of A. aegypti showing spontaneous locomotor activity was greater in low RH than in high RH, but was equal in A. triseriatus.

TABLE 1. SPIRACULAR RESPONSE TO HIGH (90±5%) AND LOW (<5%) RH IN AIR AND AIR PLUS 1/2% CARBON DIOXIDE IN 2-DAY-OLD ADULT A. AEGYPTI FOLLOWING A 5-MINUTE ACCOMMODATION PERIOD

Treatment		Freq.	toF	S.E.	toT	S.E.	Activity,[a] %	N[b]
AIR	HIGH RH	3.8	-		44.4 ±	2.3	30	30
	LOW RH	4.4	-		39.8 ±	2.5	30	30
1/2% CO_2	HIGH RH	0.5	57.6 ±	2.2	58.8 ±	1.5	53	30
	LOW RH	1.3[c]	50.5 ±	2.1[d]	53.0 ±	1.9[d]	50	30

a. Activity refers to the number of individuals showing spontaneous locomotor activity. See text for other abbreviations.
b. Number of observations.
c. P <0.005.
d. P <0.01.

TABLE 2. SPIRACULAR RESPONSE TO HIGH (90±5%) AND LOW (<5%) RH IN AIR AND AIR PLUS 1/2% CARBON DIOXIDE IN A. TRISERIATUS FOLLOWING A 5-MINUTE ACCOMMODATION PERIOD

Treatment		Freq.	toF	S.E.	toT	S.E.	Activity, %	N[a]
AIR	HIGH RH	4.5	-		43.4	± 2.3	03	30
	LOW RH	4.4	-		40.1	± 2.8	13	30
1/2% CO_2	HIGH RH	2.1	50.8	± 1.9	54.7	± 1.4	07	30
	LOW RH	2.6	40.3	± 2.5[b]	48.5	± 2.1[c]	13	30

a. Number of observations.
b. $P < 0.005$.
c. $P < 0.01$.

It was thus demonstrated that both the magnitude and duration of spiracular opening were less in a low RH than in a high RH. However, it may be argued that such a result was not a sensory response but rather a consequence of desiccation. Observations were therefore made to determine if spiracular behavior could be influenced by RH after only a 2-minute exposure period. A. aegypti 2, 3, 4, and 5 days old were randomly examined in an air stream of 90±5% RH or <5% RH. The pooled observations are shown in Table 3 and indicate that treatment means and variances were equal in both air and carbon dioxide. Relative humidity thus appeared to have no immediate sensory effect upon spiracular opening and closing in A. aegypti.

Spiracular behavior of A. triseriatus in high and low RH following 2-minute exposure periods were next measured. Additionally tested was the hypothesis that spiraculer regulation of water loss occurred only when water reserves were depleted.[3] This was done by contrasting spiracular behavior of three successive batches of newly emerged day 1 unfed insects in high and low RH. It was anticipated that the water reserves of these mosquitoes would be minimal and certainly less than in older insects given access to sucrose solution and maintained at a higher RH (sucrose-soaked cotton pads, when placed on a mosquito holding container, raise the internal RH by 10% or more). Near the end of day 1 the A. triseriatus were allowed constant access to the 10% sucrose solution provided fresh daily. They were sampled further for observations on spiracular behavior on days 2 through 4.

TABLE 3. SPIRACULAR OPENING IN A. AEGYPTI[a] IN HIGH (90±5%) AND LOW (<5%) RH

Treatment		Freq.	toF	S.E.	toT	S.E.	N[b]
AIR	HIGH RH	2.58	-		50.36 ±	2.0	50
	LOW RH	2.68	-		50.76 ±	1.8	50
1/2% CO_2	HIGH RH	0.64	55.97 ±	0.99	58.24 ±	0.57	50
	LOW RH	0.61	55.63 ±	0.86	58.67 ±	0.24	50

a. Insects were subjected to a 2-minute accommodation period in the experimental conditions prior to observation.
b. Number of observations.

Examination of the pooled observations (Table 4) indicated that in both the starved and fed mosquitoes, spiracular opening was inhibited to a highly significant extent in air of low RH. Further spiracular opening in the starved insects was considerably less than in the older, fed mosquitoes regardless of humidity. On a daily basis (Fig. 1), the average duration of spiracular opening (toT) was equal between treatments only on day 2; this may well have been the result of a particularly heavy and uniform feeding by formerly starved mosquitoes.

Spiracular response to 1/2% carbon dioxide was severely attenuated in low RH (Table 4). This was particularly so in the starved group, for here the duration of spiracular opening in low RH was 76% of that in the high RH. In the same insects, amplitude of opening was 50% of that in high RH. In the fed group of mosquitoes exposed to low RH, duration of opening was 88% and amplitude 78% of those exposed to the high RH. It is therefore apparent that low RH primarily affects the amplitude of spiracular opening. It is worthwhile to examine spiracular response to 1/2% carbon dioxide as a function of sample age (Fig. 1). As noted previously, spiracular opening was most extensive on the day following feeding of the experimental population; beyond day 2, duration and amplitude of spiracular opening progressively declined. Nevertheless, the influence of RH on toF and toT was highly significant (P <0.005) each day of sampling.

TABLE 4. SPIRACULAR BEHAVIOR OF A. TRISERIATUS[a] IN AIR AND AIR PLUS 1/2% CARBON DIOXIDE AT HIGH (90±5%) AND LOW (<5%) RH

Treatment & Status		Air		Air Plus 1/2% CO_2			
		Freq. ± S.E.	toT ± S.E.	Freq. ± S.E.	toF ± S.E.	toT ± S.E.	N[b]
Day 1 Starved	HIGH RH	4.6 ± 0.58	40.9 ± 2.4	1.4 ± 0.34	47.3 ± 1.3	54.8 ± 1.5	30
	LOW RH	5.1 ± 0.52	34.3 ± 2.1[c]	4.1 ± 0.55[c]	23.8 ± 2.9[c]	41.8 ± 1.4[c]	30
Days 2, 3, 4. Fed	HIGH RH	2.8 ± 0.20	49.0 ± 1.5	0.9 ± 0.16	52.9 ± 1.2	56.4 ± 0.97	90
	LOW RH	3.6 ± 0.27	42.9 ± 1.6[c]	2.4 ± 0.23[c]	41.5 ± 1.9[c]	49.5 ± 1.2[c]	90

a. Observations were made following a 2-minute exposure to the desired humidity.
b. Number of observations.
c. $P < 0.005$.

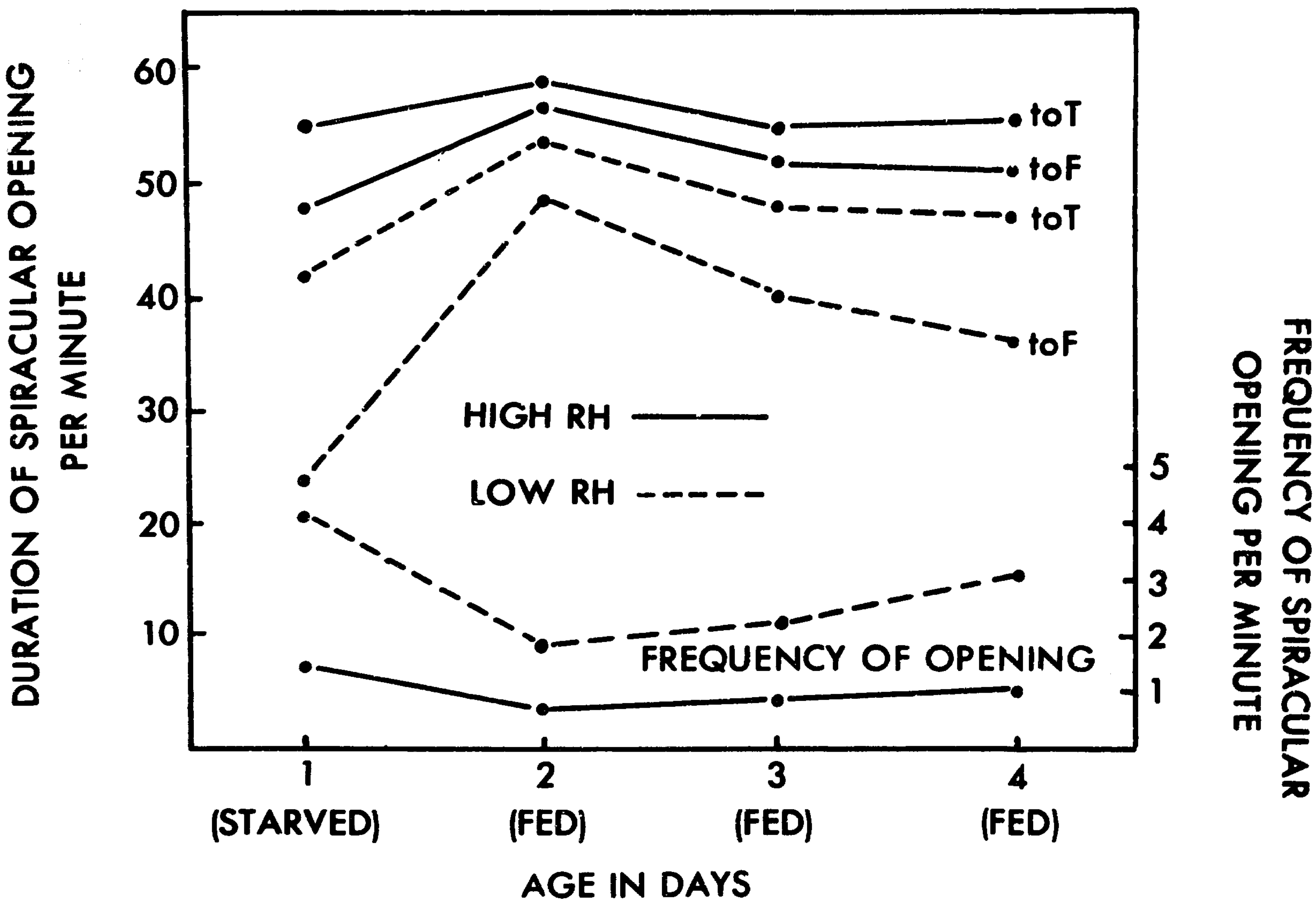

FIGURE 1. Spiracular Responses of A. triseriatus to 1/2% Carbon Dioxide in High (90±5%) and Low (<5%) RH as Functions of Sample Age. Each point is the average of 30 two-minute observations.

Immediately following the 2-minute exposure to carbon dioxide, occasional mosquitoes were subjected to a reversal in RH; this was easily accomplished by turning a valve and shunting dry air and carbon dioxide directly over the mosquito for an additional 2 minutes. Following this procedure, the RH was again reversed. By then comparing the resulting oscillograms within each 2-minute period, it was readily determined that spiracular reaction to carbon dioxide was indeed modified by RH.

There was a minimum difference of 80% RH between treatments, so it was not altogether surprising that treatment effects were demonstrated in spiracular behavior. The next objective was therefore to contrast spiracular activity in high RH and a more moderate 50±6% RH. Day 3, 4, and 5 adult female A. triseriatus were randomly exposed to one of the two humidities for 2 minutes prior to recording spiracular activity. The results are shown in Table 5 and strongly suggest that marked reduction in spiracular opening may occur even in a "moderate" RH.

TABLE 5. SPIRACULAR BEHAVIOR OF A. TRISERIATUS[a] IN AIR AND AIR PLUS 1/2% CARBON DIOXIDE OF HIGH (90±5%) AND MODERATE (50±6%) RH

Treatment		Freq. S.E.	toF S.E.	toT S.E.	N[b]
AIR	HIGH RH	3.5 ± 0.32	-	42.4 ± 2.3	50
	MODERATE RH	4.0 ± 0.26	-	36.9 ± 2.0[c]	50
1/2% CO_2	HIGH RH	1.4 ± 0.25	47.0 ± 2.0	53.8 ± 1.4	50
	MODERATE RH	3.0 ± 0.29	30.0 ± 2.4[c]	44.4 ± 1.8[c]	50

a. Mosquitoes were exposed to the experimental conditions 2 minutes prior to recording spiracular activity.
b. Number of observations.
c. $P < 0.005$.

IV. DISCUSSION

Earlier work has clearly demonstrated that spiracles greatly facilitate water conservation in insects.[2-4,9,14] Perhaps this may best be illustrated in an insect showing discontinuous respiration - in other words, respiration in "slow motion." Saturniid pupae in dry atmospheres lose through spiracular transpiration about as much water as metabolically produced carbon dioxide; however, the source of this water is oxidized fat and consequently no net loss of water occurs.[14] Kanwisher's fantastically sensitive diaferometric techniques indicated that most transpiration occurred during the "burst" period of spiracular opening. Permanently open spiracles allowed transpiration to occur at a 25-fold or even greater rate, ensuring desiccation and ultimate death.[14] In addition to control normally exerted by intratracheal pressures of oxygen and carbon dioxide, spiracular behavior may also be governed to some degree by the water reserves of the insect. There is good evidence that spiracular control of water loss through transpiration rests critically upon water balance in Glossina[3] and in dragonflies.[9] By measuring the rate of water loss of tsetse flies in varying states of desiccation, Bursell was able to show that spiracular control of transpiration increased as water reserves decreased. It was also demonstrated that transpiration in normal flies was not related to the ambient RH, with the conclusion that decreasing humidity increased the degree of spiracle closure.[3] In dragonflies, desiccation results in a greater concentration of carbon dioxide necessary to elicit spiracular opening;[9] hydration has the opposite effect.

This study demonstrates that, quite apart from the intrinsic effect of water balance upon the degree of spiracular control, RH itself exerts a direct, probably sensory, effect upon the thoracic spiracles of A. aegypti and especially A. triseriatus. Thus, both spiracular behavior in air and spiracular response to carbon dioxide become quickly attenuated in low RH compared with that in a high RH. Moreover, this behavior may occur without regard to the mosquito's state of hydration. The principal feature of spiracular behavior in low RH was a reduction in the amplitude of opening. Secondly, duration of opening was decreased and, finally, an increased frequency of opening and closing occurred. Attempts by Geigy and Huber[12] to correlate changes in RH with the spiracular behavior of tsetse flies failed. Bursell[3] attributed this to the fact that the insects used by Geigy and Huber were given opportunity to feed daily; his own experiments were conducted exclusively with unfed flies. Thus, Bursell suggested that spiracular control of water loss occurs only when water reserves are somewhat depleted. One-day-old unfed adult Aedes showed quite clearly more conservative spiracular opening than did older, fed specimens, and the responses to low RH were certainly more exaggerated. However, 2-day-old Aedes fully replete with 10% sucrose solution also showed marked attenuation of spiracular opening in low RH. Partly for this reason, it is suggested here that such behavior in low RH is a result of a sensory process. Although it can be argued that 1-day-old Aedes may

inherently show more conservative spiracular behavior, a study of spiracular behavior as a function of age suggested quite the opposite* and it must be concluded that responses to low RH were enhanced by reduced water reserves. The "sensory" effect of RH postulated above rests also upon the observation that it could be demonstrated within 3 minutes of exposure and was reversible.

It is interesting to contrast the responses of A. aegypti and A. triseriatus to RH. In the former species, spontaneous locomotor activity was high and spiracular behavior less conservative than in the much less active A. triseriatus. It has been shown that spiracular opening is considerably exaggerated in A. aegypti, probably as a result of spontaneous activity;[13] it is suggested that this may account for the absence of a significantly different response to low RH following a 2-minute exposure. That is, the respiratory dictates of activity may have overridden the effect of RH upon spiracular behavior in A. aegypti.

* Krafsur, in preparation.

LITERATURE CITED

1. Hazelhoff, E.H. 1927. Die Regulierung der Atmung bei Inseckten und Spinnen. Z. Vergleich. Physiol. 5:179-190.

2. Mellanby, K. 1934. The site of water loss from insects. Proc. Roy. Soc. Ser. B 116:139-149.

3. Bursell, E. 1957. Spiracular control of water loss in the tsetse fly. Proc. Roy. Entomol. Soc. 32:21-29.

4. Buck, J.B.; Keister, M.L. 1949. Respiration and water loss in the adult blowfly, Phormia regina, and their relation to the physiological action of DDT. Biol. Bull. 97:64-81.

5. Bursell, E. 1963. Tsetse-fly physiology. Bull. WHO 28:703-709.

6. Clements, A.N. 1963. The physiology of mosquitoes. Pergamon Press, Inc., New York, N.Y.

7. Hylton, A.R. 1967. Low humidity water-retention ability in Eretmapodites chrysogaster and Aedes albopictus. J. Insect Physiol. 13:153-157.

8. Bursell, E. 1964. Environmental aspects: Humidity, p. 323-361. In M. Rockstein (ed.) The physiology of insecta, Vol. 1. Academic Press, New York, N.Y.

9. Miller, P.L. 1964. Factors altering spiracle control in adult dragonflies: Water balance. J. Exp. Biol. 41:331-343.

10. Miller, P.L. 1964. Factors altering spiracle control in adult dragonflies: Hypoxia and temperature. J. Exp. Biol. 41:345-357.

11. Hoyle, G. 1961. Functional contracture in a spiracular muscle. J. Insect Physiol. 7:305-314.

12. Geigy, R.; Huber, M. 1952. Untersuchungen uber Bau und Funcktion der Stigmen bei verschiedenen Glossina-Arten und bei Stomoxys calcitrans. Acta Trop. 9:233-263.

13. Krafsur, E.S.; Willman, J.R.; Graham, C.L.; Williams, R.E. March 1969. Observations on spiracular behavior in Aedes mosquitoes, (Technical Manuscript 517). Plant Pathclogy Division, Fort Detrick, Frederick, Maryland.

14. Kanwisher, J.W. 1966. Tracheal gas dynamics in pupae of the cecropia silkworm. Biol. Bull. 130:96-105.

Unclassified
Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)
Department of the Army
Fort Detrick, Frederick, Maryland, 21701

2a. REPORT SECURITY CLASSIFICATION
Unclassified

2b. GROUP

3. REPORT TITLE
SENSORY EFFECTS OF RELATIVE HUMIDITY ON THORACIC SPIRACLES OF AEDES MOSQUITOES

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

5. AUTHOR(S) (First name, middle initial, last name)
Elliot S. Krafsur

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
April 1969	19	14

8a. CONTRACT OR GRANT NO.

b. PROJECT NO. 1B562602A059

c.

d.

9a. ORIGINATOR'S REPORT NUMBER(S)
Technical Manuscript 529

9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)

10. DISTRIBUTION STATEMENT
Qualified requesters may obtain copies of this publication from DDC.
Foreign announcement and dissemination of this publication by DDC is not authorized.
Release or announcement to the public is not authorized.

11. SUPPLEMENTARY NOTES

12. SPONSORING MILITARY ACTIVITY
Department of the Army
Fort Detrick, Frederick, Maryland, 21701

13. ABSTRACT

The behavior of the thoracic spiracles of *A. triseriatus* and *A. aegypti* was observed in high (90-5%) and low (<5%) relative humidities. Following a 5-minute exposure to low RH, spiracular responses to 1/2% carbon dioxide were reduced in both species, but only *A. triseriatus* showed attenuated spiracular behavior after a 2-minute exposure. Conservative spiracular opening was observed in both unfed and fed mosquitoes. It is postulated that the spiracular response to low RH is a sensory one.

14. Key Words
*Aedes
*Culicidae
*Humidity
Thorax
Spiracles
Senses
Sensitivity
Behavior
Aedes triseriatus
Aedes aegypti

DD FORM 1 NOV 65 1473 REPLACES DD FORM 1473, 1 JAN 64, WHICH IS OBSOLETE FOR ARMY USE.

Unclassified
Security Classification